Journal of Forensic and Legal Medicine 18 (2011) 52–56

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original Communication

Postmortem degradation of porcine articular cartilage

Christopher J. Rogers MSc Student [a,b], Kirsty Clark BSc (Hons) Student [a,b], Barbara J. Hodson Technical support [a,b], Michael P. Whitehead PhD Lecturer [a,b], Raul Sutton PhD Lecturer [a,b,*], Wera M. Schmerer Dr rer nat, Lecturer [a,b]

[a] Department of Forensic and Molecular Science, University of Wolverhampton, Wulfruna Street, Wolverhampton WV5 0LW, United Kingdom
[b] Research Centre in Applied Sciences. University of Wolverhampton, WV1 1LY, United Kingdom

ARTICLE INFO

Article history:
Received 25 February 2010
Received in revised form
4 November 2010
Accepted 24 November 2010
Available online 31 December 2010

Keywords:
Articular cartilage
Decomposition
Postmortem interval

ABSTRACT

Postmortem decompositional changes to articular cartilage were analysed to help establish a new methodology in determining the postmortem interval. The cartilage was collected from porcine trotters buried in simulated shallow graves for different time periods. The trotters were dissected to expose the cartilage located at the metatarsal joint. Numerous macroscopic changes including a colour change, gradual degradation of cartilage and adjacent soft tissues and a loss of cartilage covering articular facets were observed. Further analysis was conducted using light microscopy (LM) and scanning electron microscopy (SEM) to assess microstructural changes. Both LM and SEM showed gradual morphological and structural changes to the tissue over time, along with loss of nuclear material. Tissue surface analysis with SEM highlighted orthorhombic shaped crystals that appear at approximately three weeks postmortem and persist until six weeks postmortem. Both microscopic and macroscopic characteristics followed a recognisable succession over the burial times observed. These results indicate that postmortem degradation of articular cartilage may be useful for estimating a presumptive postmortem interval.

1. Introduction

The term taphonomy, meaning the 'law of burial', was first introduced by Efremov,[1] and includes expertise from academic fields such as; palaeontology, archaeology, and, more recently, mycology, botany, entomology and forensic science.[2,3] 'Forensic taphonomy' is concerned with understanding decomposition processes and the factors that influence them, as relevant in a legal context.[4] Forensic taphonomy has two branches, biotaphonomy, which is directly concerned with the corpse postmortem and geotaphonomy, which is concerned with the act of burial itself, together with the interactions between the environment and the corpse.[5] Sledzik[6] states that one way to imagine decomposition is as a linear progression, and at points along this line different scientific methods are employed to determine how much time has elapsed since death. Numerous methods are currently utilised for the determination of the postmortem interval (PMI). Notable methods include temperature loss,[7] forensic entomology,[8] analysis of volatile fatty acid production,[9] and the use of radionuclides.[10–12] However, it is widely regarded that as the time elapsed since death increases, the overall accuracy of PMI determination using current methodologies decreases.[13]

Postmortem changes to cartilage have received very little research which is restricted to chondrocyte loss.[14] Cartilage is particularly useful for postmortem analysis because the tissue possesses a low cell density.[15,16] This may delay autolytic processes and, as articular cartilage is internal to the subject, it is relatively shielded from external influences such as scavenger activity and contamination by foreign materials. It is also reported to be protected from putrefaction due to its compartmentalisation from other tissues.[14]

The methods presented here detail how macroscopic and microscopic analysis of articular cartilage can help to establish the PMI for remains that have been buried for up to 13 weeks. Due to the similarities between porcine and human samples in aspects of body composition,[17,18] porcine hind trotters were used as human analogues for this research. Porcine substitutes are common in forensic experiments into decomposition.[19]

2. Methodology

Porcine hind trotters were interred in soil to a depth of 20 cm, at the University of Wolverhampton's Crop Technology Unit, Compton Campus. The soil type at this site is sandy silt loam[20] with an average pH of 5.5. The experiments occurred during both winter to

* Corresponding author. Department of Forensic and Molecular Science, University of Wolverhampton, Wulfruna Street, Wolverhampton WV5 0LW, United Kingdom. Tel.: +44 1902 322167.
E-mail address: R.Sutton@wlv.ac.uk (R. Sutton).

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doi:10.1016/j.jflm.2010.11.006

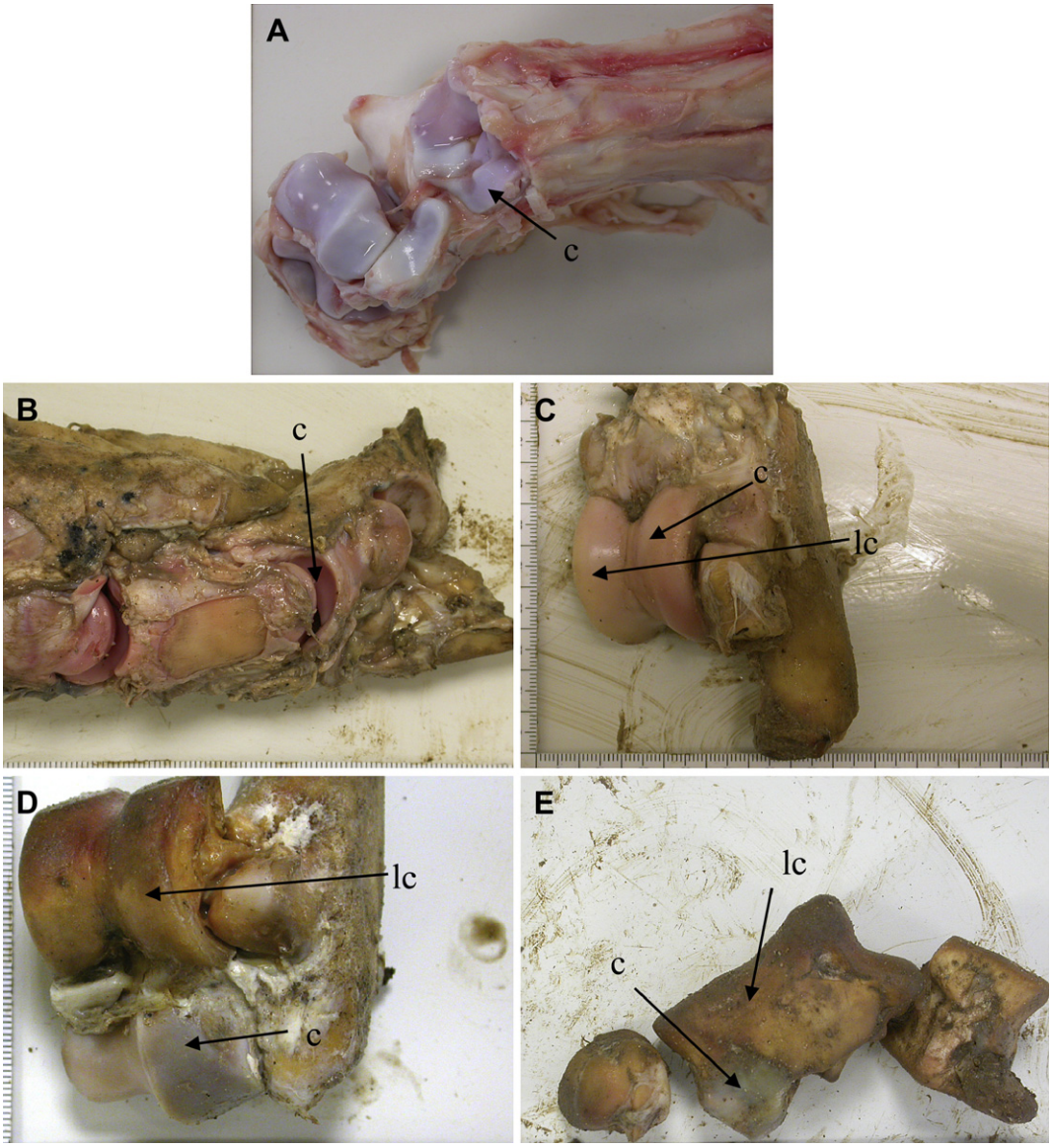

Fig. 1. Macroscopic analysis of articular cartilage. A, B, C, D and E refers to 0, 4, 7, 10 and 13 weeks respectively. c in the images refers to cartilage and lc to areas of cartilage loss.

spring (November to March) and spring to summer (February to June) over three years. Excavation occurred at various intervals until skeletonisation was observed. During sample excavation soil was removed in small layers until the trotters were exposed. The samples were lifted (sometimes block lifted in soil to prevent damage to the sample), placed in sealed plastic containers and stored at −20 °C to prevent further degradation. A total of 15 replicates (Seven and eight replicates in the summer and winter respectively) were conducted which equated to 30 trotters for each postmortem interval analysed.

The entire trotter was subjected to macroscopic analysis to record any visually discernable changes in overall appearance (Coolpix 4500, Nikon). Analysis of the cartilage at the macroscopic level allowed for the application of a grading system as first proposed by Mcllwraith et al.,[21] and later modified by Fuller et al.,.[22] Cartilage was dissected taking care to ensure the articular surface was identifiable.

SEM analysis of dissected cartilage was conducted with an EVO 50 Scanning Electron Microscope (Zeiss) in the cool stage (Deben). This ensured that the samples did not thaw during the analysis which might have altered the tissue structure. The samples were mounted on carbon stubs with the articular surface facing up. Scanning electron microscopy coupled with energy dispersive x-ray (SEM-EDX) was used to analyse the chemical composition of surface crystals.

Table 1
Summary of macroscopic findings for articular cartilage postmortem.

Burial time (Weeks)	Colour	Coverage	Texture	Score ()/Degree of articular damage. After Fuller *et al*[22]
0 (control)	Intense white and purple	Complete	Very smooth & moist	(0) no damage
4	Deep pink	Complete	Smooth	(1) minimal damage
7	Pale pink	Partial	Smooth	(2) damage to ∼ 30% of cartilage
10	Dull cream	Partial	Smooth	(3) loss of 50% of cartilage
13	Dull cream	Little	Soft	(4) severe loss of cartilage (>50%)

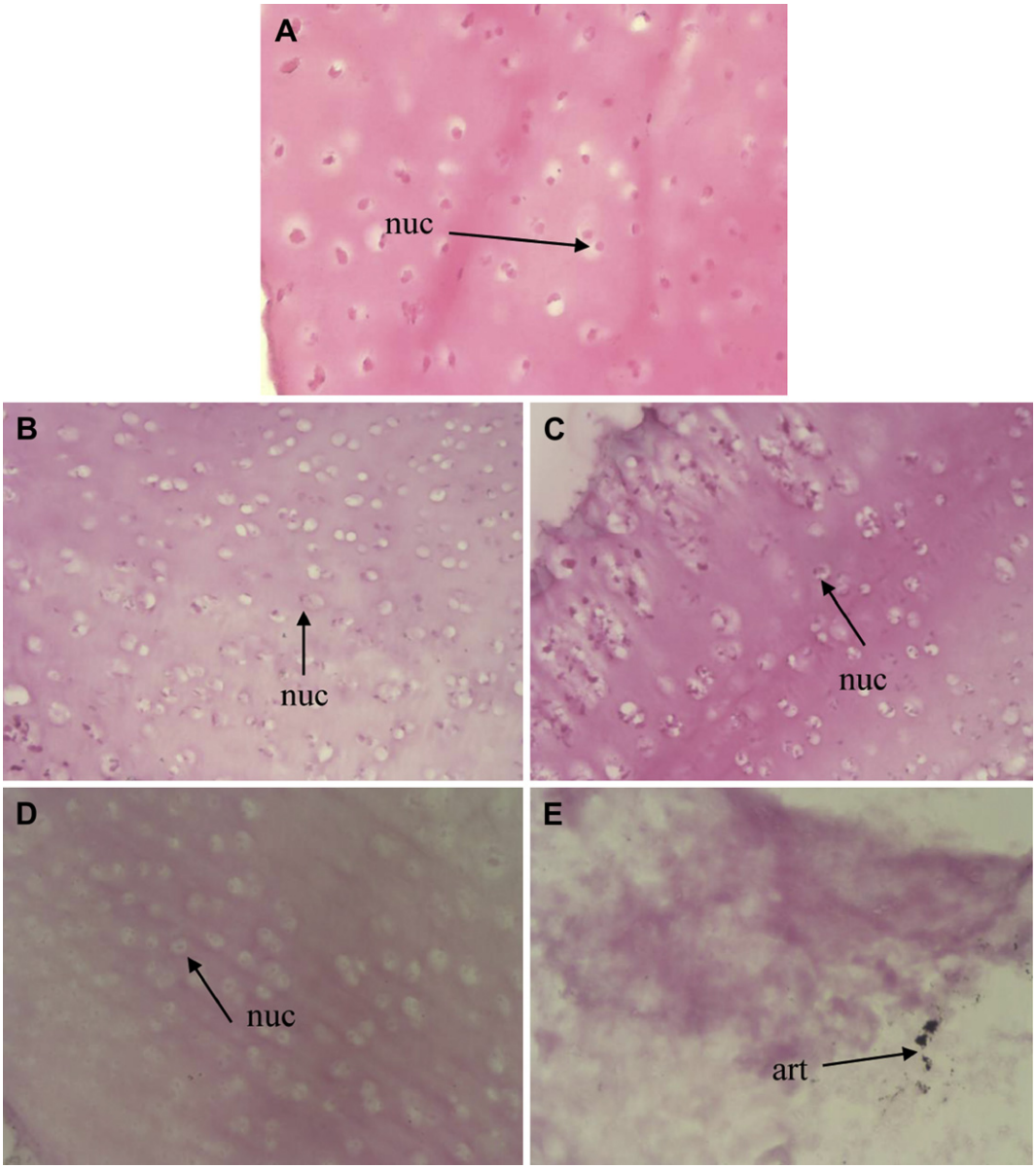

Fig. 2. Microscopic analysis of articular cartilage. Histological sections stained with Haematoxylin and Eosin 400× magnification. A, B, C, D and E refers to 0, 3, 6, 9 and 12 weeks. nuc = nuclear material and art = non relevant artefacts.

Cartilage samples were sectioned using a cryostat (Cryocut-E, Reichert-Jung) set to maintain a temperature of −20 °C. The sample was embedded in 70% gelatine, frozen in liquid nitrogen and sectioned between 7 and 12 μm.

Haematoxylin and Eosin (H&E) was used, which stains cell nuclei dark pink to blue.[23] After the stain had been applied, the samples were fixed in polystyrene and xylene (DPX) and covered with a standard microscope cover slip. Light microscopic analysis (at 400× magnification) was conducted using an Eclipse E600 (Nikon) with a camera attachment.

3. Results

Fig. 1 shows the results of the macroscopic examination of cartilage following burial between 0 and 13 weeks, after 13 weeks no cartilage was visible. The trotters, as whole specimens, demonstrated a gradual degradation over time, showing successive changes in colour, texture and the amount of remaining cartilage, ending with partial or complete skeletonisation. The changes are summarised in Table 1

Fig. 1A shows the cartilage to exhibit a healthy, white colour with areas of translucency resulting in the emergence of a pink colour. The white colour is gradually lost after four weeks (Fig. 1B), and changes to a deeper pink. After seven weeks areas of cartilage loss results in exposed subchondral bone but with the remaining cartilage still pink (Fig. 1C). At 10 weeks there was further cartilage loss and any remaining cartilage has turned a dull cream colour (Fig. 1D). Finally, at 13 weeks there was a dramatic loss of soft tissues resulting in exposed articular surfaces with only small patches of cartilage remaining (Fig. 1E).

Samples for microscopic analysis were buried for between 0 and 12 weeks. The cartilage was sectioned and stained with Haematoxylin and eosin and shows a progressive loss of nuclear material over time (Fig. 2).

This progressive loss of nuclei was represented in all replicates analysed. The three week sample (Fig. 2A) was similar in appearance

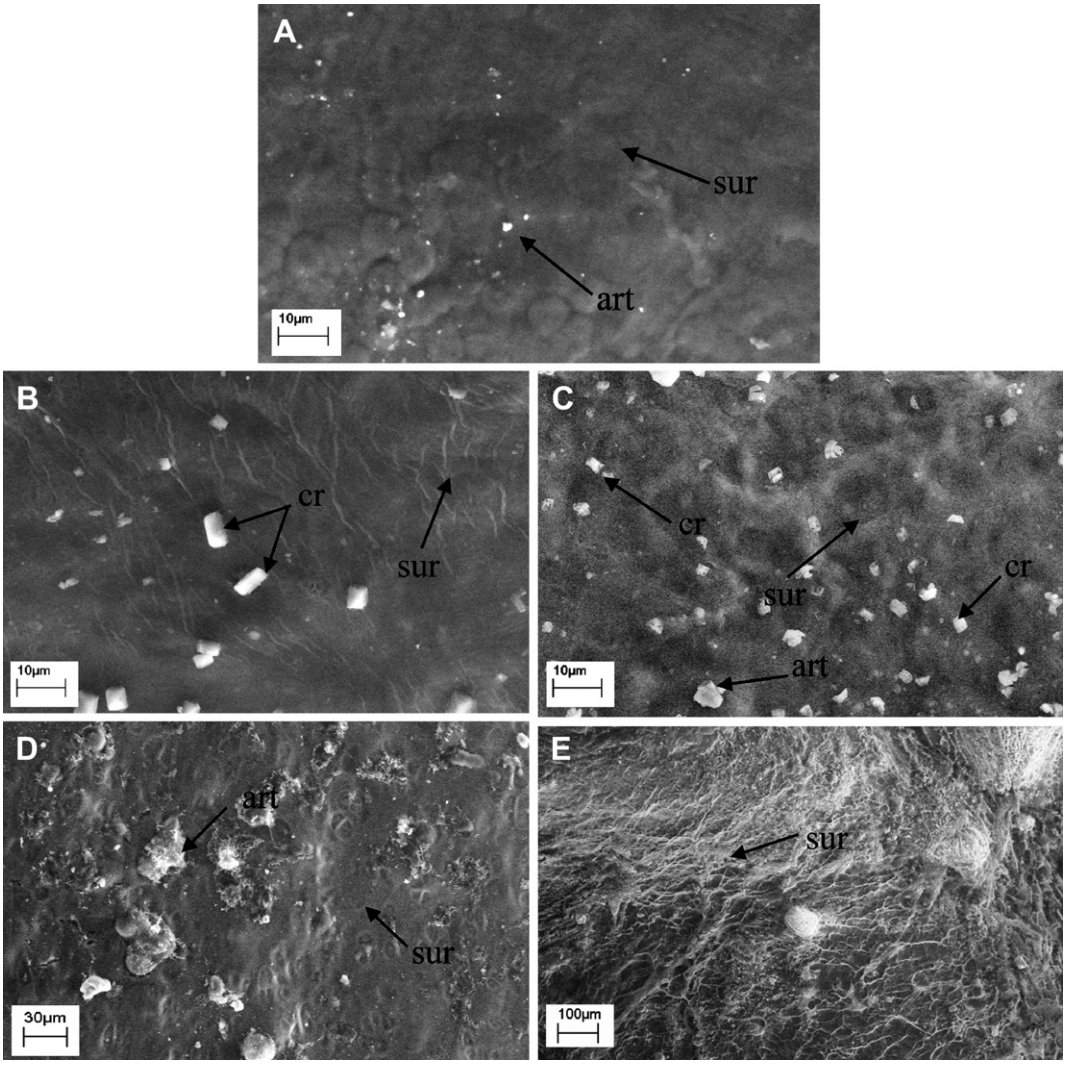

Fig. 3. Scanning electron micrographs of articular cartilage from buried samples. A, B, C, D and E refers to 0, 3, 6, 9 and 12 weeks. Art refers to an artefact, sur to the cartilage surface and cr to a crystal.

to the control. The dye intensity was strong and the sample was architecturally sound, furthermore nuclear material was in abundance. The six week sample (Fig. 2C) was, morphologically similar. The nine week sample (Fig. 2D) began to exhibit signs of degradation, the dye intensity was reduced as was the level of nuclear material. The overall structure was beginning to break down with large open spaces forming. The 12 week sample (Fig. 2E) exhibited complete degradation with no recognisable cartilage features.

Additional pieces of cartilage collected from the same trotters used for the Haematoxylin and Eosin analysis were analysed with SEM which detailed changes on the cartilage surface (Fig. 3).

The control cartilage (Fig. 3A) has a smooth and intact surface with no evidence of degradation. At three weeks postmortem (Fig. 3B) orthorhombic crystals were observed, the overall cartilage structure was smooth but scoring was apparent. Artefacts were differentiated from crystals by SEM-EDX. At six weeks (Fig. 3C) the surface exhibited undulations with possible exposure of subsurface lacunae. Furthermore, crystals were still observable. At nine weeks (Fig. 3D) the crystals were usually absent and the surface of the tissue was pitted and eroded. The 12 week sample (Fig. 3E) never exhibited any crystals and the tissue was extensively degraded, exposing a fibrous network.

Table 2 summarises the major changes that were seen with the LM and SEM analysis. To determine if contact with soil was responsible for crystal formation trotters were stored in plastic containers placed outside during the summer. Crystals were also found to form. Fig. 4 shows the presence of crystals after seven days (July).

Table 2
Major changes observed during analysis of cartilage with LM and SEM.

Sample	H&E nuclear material	SEM surface degradation	SEM Presence of crystals
0 weeks (control)	Abundant	None	Absent
3 Week	Abundant	Slight scoring	Present – numerous
6 Week	Abundant	Mild, exposure of subsurface lacunae	Present
9 Week	Sparse	Eroded	None
12 Week	Absent	Heavily damaged–fibrous like appearance	None

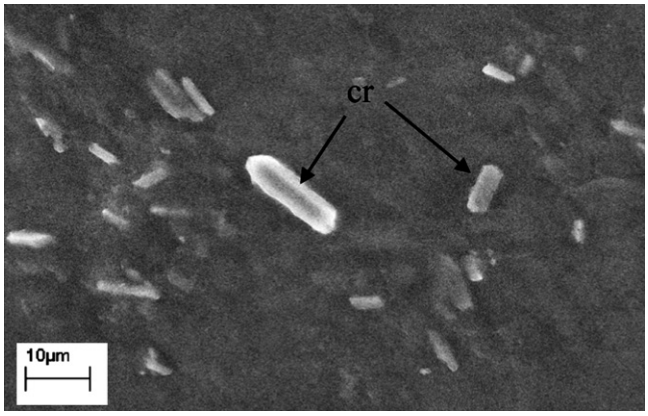

Fig. 4. Scanning electron micrograph of articular cartilage from a sample not buried in soil. cr refers to crystals.

4. Discussion

Obvious ordered degradational changes in cartilage can be observed as the postmortem interval increased. Little variation (+/− three days) in decomposition was observed between the winter and summer months. Visible macroscopic observations included a change in colour (initially from white to a dull cream), together with a loss in cartilage covering. H&E staining illustrated the loss of nuclear material over time with the greatest loss between six and nine weeks. This is consistent with the findings of Lasczkowski et al.,[14] who documented a loss of chondrocytes as the PMI increased. Cartilage became unrecognisable at 12 weeks postmortem where it is degraded to an extent where it is of no value in this context.

This paper provides preliminary data suggesting that cartilage analysis can be used for determining PMI. At this stage valuable information has been presented to recommend that this method become a viable preliminary methodology for determining the postmortem interval. The methodology could be used in conjunction with other methods.[8,24] However, there is a dearth of methods available for determining PMI between a few days and several months especially in buried environments. Utilisation of this method may help narrow down the PMI to a range of zero to 12 weeks with techniques employed by most forensic services. SEM analysis illustrated the presence of crystals seen on the cartilage surface at approximately three to six weeks postmortem, their presence is consistent in all replicates conducted, both in winter and summer. Further experiments in the absence of soil showed that these crystals still form, consequently their formation is not soil dependent. The crystals are currently undergoing identification (Rogers et al. in preparation).

There are limitations to the application of this data (as with all taphonomic experiments) such as environmental influences, namely temperature and soil type, which can result in different decomposition rates. This means that the data presented here may not be directly applicable to other geographical locations with different soil types or climatic conditions. It is also important to apply this method to human remains to see if the same processes occur. If so, then results from this research can be of use to forensic pathologists and anthropologists who routinely analyse remains in various stages of decomposition. Furthermore, application of this method to other animal species may be beneficial to determine PMI in wildlife forensic cases e.g. poaching. The methods utilised during this research provided new information exploring how cartilage decomposes in a burial environment.

Acknowledgments

We thank the Research Centre in Applied Sciences (RCAS), University of Wolverhampton for fully funding this research program. Thank you to Phil Warren for his help with histology. To Dr. Malcolm Inman for his endless help with microscopy. To Robert Hooton for his help in preparing the Compton site and finally to Danen Appasamy for his help with editing.

Conflict of interest
None declared.

Funding
None declared.

Ethical approval
None declared.

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